CLAIMS

We claim:

1. A method for identifying an agent that alters mitochondrial ATP production, comprising:

comparing (i) a level of binding of an endogenous inhibitor of ATP synthase to an ATP synthase subunit in the presence of a candidate agent to (ii) the level of binding of an endogenous inhibitor of ATP synthase to an ATP synthase subunit in the absence of the candidate agent, wherein an altered level of binding indicates that the agent alters mitochondrial ATP production.

2. The method according to claim 1 wherein the endogenous inhibitor of ATP synthase is an IF1.

3. The method according to claim 2 wherein the IF1 is a mammalian IF1.

4. The method according to claim 3 wherein the mammalian IF1 is selected from the group consisting of a mouse IF1, a rat IF1, a rabbit IF1, a bovine IF1, a canine IF1, a non-human primate IF1 and a human IF1.

5. The method according to claim 2 wherein the IF1 comprises a portion of an IF1 polypeptide, said portion comprising a polypeptide of less than 35 amino acids.

6. The method according to claim 5 wherein the portion of an IF1 polypeptide comprises a polypeptide selected from the group consisting of the IF1 fragment 14-47 set forth in SEQ ID NO:29, the IF1 fragment 14-46 set forth in SEQ ID NO:67, the IF1 fragment 14-45 set forth in SEQ ID NO:66, the IF1 fragment 14-44 set forth in SEQ ID

NO:65, the IF1 fragment 14-43 set forth in SEQ ID NO:64 and the IF1 fragment 14-42 set forth in SEQ ID NO:63.

7. The method according to claim 5 wherein the portion of the IF1 polypeptide comprises IF1 fragment 14-47 (SEQ ID NO:29).

8. A method for identifying an agent that alters mitochondrial ATP production, comprising:

contacting, in the absence and presence of a candidate agent, an isolated IF1 polypeptide and an isolated mitochondrial ATP synthase, wherein the ATP synthase is capable of ATP synthesis, under conditions and for a time sufficient for ATP production to occur; and

comparing a level of ATP production by the ATP synthase in the presence of the candidate agent to a level of ATP production in the absence of the candidate agent, and therefrom identifying an agent that alters mitochondrial ATP production.

9. The method according to claim 8 wherein the IF1 comprises a portion of an IF1 polypeptide, said portion comprising a polypeptide of less than 35 amino acids.

10. The method according to claim 9 wherein the portion of an IF1 polypeptide comprises a polypeptide selected from the group consisting of the IF1 fragment 14-47 set forth in SEQ ID NO:29, the IF1 fragment 14-46 set forth in SEQ ID NO:67, the IF1 fragment 14-45 set forth in SEQ ID NO:66, the IF1 fragment 14-44 set forth in SEQ ID NO:65, the IF1 fragment 14-43 set forth in SEQ ID NO:64 and the IF1 fragment 14-42 set forth in SEQ ID NO:63.

11. The method according to claim 9 wherein the portion of an IF1 polypeptide comprises IF1 fragment 14-47 (SEQ ID NO:29).

12. The method according to claim 8 wherein the isolated mitochondrial ATP synthase is part of a submitochondrial particle or an alkaline submitochondrial particle.

13. A method for treating diabetes comprising administering to a patient in need thereof an effective amount of a compound that (a) increases the synthesis of mitochondrial ATP in cells, (b) decreases the hydrolysis of mitochondrial ATP in cells, or (c) does both (a) and (b).

14. The method according to claim 13, wherein the compound is selected from the group consisting of a composition that inhibits one or more activities of IF1 and a composition that mimics IF1.

15. The method according to claim 14 wherein the composition that mimics IF1 comprises a portion of an IF1 polypeptide, said portion comprising a polypeptide of less than 35 amino acids.

16. The method according to claim 15 wherein the portion of an IF1 polypeptide comprises a polypeptide selected from the group consisting of the IF1 fragment 14-47 set forth in SEQ ID NO:29, the IF1 fragment 14-46 set forth in SEQ ID NO:67, the IF1 fragment 14-45 set forth in SEQ ID NO:66, the IF1 fragment 14-44 set forth in SEQ ID NO:65, the IF1 fragment 14-43 set forth in SEQ ID NO:64 and the IF1 fragment 14-42 set forth in SEQ ID NO:63.

17. The method according to claim 15 wherein the portion of an IF1 polypeptide comprises IF1 fragment 14-47 (SEQ ID NO:29).

18. The method according to claim 15 wherein the composition that mimics IF1 comprises a fusion protein, the fusion protein comprising at least one of an optional epitope tag, a cellular transport sequence, and an organellar targeting sequence.

19. The method according to claim 18 wherein the fusion protein comprises an amino acid sequence as set forth in SEQ ID NO:71.

20. A method for identifying an agent useful for treating diabetes, comprising comparing (i) a level of ATP in a biological sample comprising at least one mitochondrion before contacting the sample with a candidate agent, to (ii) the level of ATP in the sample after contacting the sample with the candidate agent, wherein an increased level of ATP indicates the agent is useful for treating diabetes.

21. The method according to claim 20 wherein the level of ATP in the sample is an intramitochondrial level of ATP.

22. A method for identifying an agent that alters glucose homeostasis, comprising:

(a) contacting a first biological sample comprising an insulin producing cell with a candidate agent and a second biological sample comprising an insulin producing cell with an IF1 polypeptide, in the presence of glucose and for a time sufficient for the cells to secrete insulin;

(b) measuring an amount of glucose stimulated insulin secretion (GSIS) in each of the first and second biological samples; and

(c) comparing the amount of GSIS in the first biological sample to the amount of GSIS in the second biological sample to detect an effect of the candidate agent on GSIS that mimics the effect of the IF1 polypeptide on GSIS, and therefrom identifying an agent that alters glucose homeostasis.

23. The method according to claim 22 wherein the insulin producing cell is INS-1.

24. The method according to claim 22 wherein the IF1 comprises a portion of an IF1 polypeptide, said portion comprising a polypeptide of less than 35 amino acids.

25. The method according to claim 24 wherein the portion of an IF1 polypeptide comprises a polypeptide selected from the group consisting of the IF1 fragment 14-47 set forth in SEQ ID NO:29, the IF1 fragment 14-46 set forth in SEQ ID NO:67, the IF1 fragment 14-45 set forth in SEQ ID NO:66, the IF1 fragment 14-44 set forth in SEQ ID NO:65, the IF1 fragment 14-43 set forth in SEQ ID NO:64 and the IF1 fragment 14-42 set forth in SEQ ID NO:63.

26. The method of claim 24 wherein the portion of an IF1 polypeptide comprises IF1 fragment 14-47 (SEQ ID NO:29).

27. The method of claim 22 wherein the IF1 polypeptide comprises a fusion protein, the fusion comprising (i) an optional epitope tag fused to (ii) a cellular transport sequence fused to (iii) an organellar targeting sequence fused to (iv) an IF1 polypeptide.

28. The method of claim 27 wherein the optional epitope tag comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, and 68.

29. The method of claim 27 wherein the optional epitope tag comprises a polyhistidine tag.

30. The method of claim 29 wherein the polyhistidine tag comprises an amino acid sequence as set forth in SEQ ID NOS:1 or 68.

31. The method of claim 27 wherein the cellular transport sequence comprises a tat sequence.

32. The method of claim 31 wherein the tat sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 10, 27 and 70.

33. The method of claim 27 wherein the organellar targeting sequence comprises a mitochondrial targeting sequence.

34. The method of claim 33 wherein the mitochondrial targeting sequence comprises an amino acid sequence as set forth in SEQ ID NO:69.

35. The method of claim 27 wherein the IF1 polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS:12, 13, 29, 63, 64, 65, 66 and 67.

36. The method according to claim 27 wherein the optional epitope tag comprises a polyhistidine tag having an amino acid sequence as set forth in SEQ ID NOS:1 or 68, the cellular transport sequence comprises a tat sequence as set forth in SEQ ID NO:70, the organellar targeting sequence comprises a mitochondrial targeting sequence as set forth in SEQ ID NO:69, and the IF1 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:29.

37. The method according to claim 27 wherein the fusion comprises an amino acid sequence as set forth in SEQ ID NO:71.

38. A method for identifying an agent that alters mitochondrial ATP hydrolase activity, comprising:

(a) contacting a first biological sample comprising an isolated mitochondrial ATP synthase with a candidate agent and a second biological sample comprising an isolated mitochondrial ATP synthase with an IF1 polypeptide, under conditions and for a time sufficient for mitochondrial ATP hydrolase activity to occur;

(b) measuring a level of mitochondrial ATP hydrolase activity in each of the first and second biological samples; and

(c) comparing the level of mitochondrial ATP hydrolase activity in the first biological sample to the level of mitochondrial ATP hydrolase activity in the second biological sample to detect an effect of the candidate agent on mitochondrial ATP hydrolase activity that mimics the effect of the IF1 polypeptide on ATP hydrolase activity, and therefrom identifying an agent that alters mitochondrial ATP hydrolase activity.

39. The method according to claim 38 wherein the IF1 comprises a portion of an IF1 polypeptide, said portion comprising a polypeptide of less than 35 amino acids.

40. The method according to claim 39 wherein the portion of an IF1 polypeptide comprises a polypeptide selected from the group consisting of the IF1 fragment 14-47 set forth in SEQ ID NO:29, the IF1 fragment 14-46 set forth in SEQ ID NO:67, the IF1 fragment 14-45 set forth in SEQ ID NO:66, the IF1 fragment 14-44 set forth in SEQ ID NO:65, the IF1 fragment 14-43 set forth in SEQ ID NO:64 and the IF1 fragment 14-42 set forth in SEQ ID NO:63.

41. The method according to claim 39 wherein the portion of an IF1 polypeptide comprises IF1 fragment 14-47 (SEQ ID NO:29).

42. The method according to claim 38 wherein the IF1 polypeptide comprises a fusion protein as set forth in (SEQ ID NO:71).

43. The method according to claim 38 wherein the isolated mitochondrial ATP synthase is part of a submitochondrial particle or an alkaline submitochondrial particle.

44. The method according to claim 38 wherein the mitochondrial ATP hydrolase activity is inhibited.

45. A method for identifying an agent for treating diabetes, comprising:

(a) contacting a first biological sample comprising an isolated mitochondrial ATP synthase with a candidate agent and a second biological sample comprising an isolated mitochondrial ATP synthase with an IF1 polypeptide, under conditions and for a time sufficient for mitochondrial ATP hydrolase activity to occur;

(b) measuring a level of mitochondrial ATP hydrolase activity in each of the first and second biological samples; and

(c) comparing the level of mitochondrial ATP hydrolase activity in the first biological sample to the level of mitochondrial ATP hydrolase activity in the second biological sample to detect an effect of the candidate agent on mitochondrial ATP hydrolase activity that mimics the effect of the IF1 polypeptide on ATP hydrolase activity, and therefrom identifying an agent for treating diabetes.

46. The method according to claim 45 wherein the IF1 comprises a portion of an IF1 polypeptide, said portion comprising a polypeptide of less than 35 amino acids.

47. The method according to claim 46 wherein the portion of an IF1 polypeptide comprises a polypeptide selected from the group consisting of the IF1 fragment

14-47 set forth in SEQ ID NO:29, the IF1 fragment 14-46 set forth in SEQ ID NO:67, the IF1 fragment 14-45 set forth in SEQ ID NO:66, the IF1 fragment 14-44 set forth in SEQ ID NO:65, the IF1 fragment 14-43 set forth in SEQ ID NO:64 and the IF1 fragment 14-42 set forth in SEQ ID NO:63.

48. The method according to claim 46 wherein the portion of an IF1 polypeptide comprises IF1 fragment 14-47 (SEQ ID NO:29).

49. The method according to claim 45 wherein the IF1 polypeptide comprises a fusion protein as set forth in (SEQ ID NO:71).

50. The method according to claim 45 wherein the isolated mitochondrial ATP synthase is part of a submitochondrial particle or an alkaline submitochondrial particle.

51. The method according to claim 45 wherein the mitochondrial ATP hydrolase activity is inhibited.

52. An agent identified according to any one of the methods of claims 1, 8, 20, 22, 38, or 45.

53. A fusion protein comprising an optional epitope tag fused to an IF1 polypeptide.

54. The fusion protein of claim 53 wherein the optional epitope tag comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, and 68.

55. The fusion protein of claim 53 wherein the optional epitope tag comprises a polyhistidine tag.

56. The fusion protein of claim 53 wherein the polyhistidine tag comprises an amino acid sequence as set forth in SEQ ID NOS:1 or 68.

57. The fusion protein of claim 53 wherein the IF1 polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS:12, 13, 29, 63, 64, 65, 66 and 67.

58. A fusion protein comprising (i) an optional epitope tag, which is fused to (ii) a cellular transport sequence, which is fused to (iii) an organellar targeting sequence, which is fused to (iv) an IF1 polypeptide.

59. The fusion protein of claim 58 wherein the optional epitope tag comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, and 68.

60. The fusion protein of claim 58 wherein the optional epitope tag comprises a polyhistidine tag.

61. The fusion protein of claim 60 wherein the polyhistidine tag comprises an amino acid sequence as set forth in SEQ ID NOS:1 or 68.

62. The fusion protein of claim 58 wherein the cellular transport sequence comprises a tat sequence.

63. The fusion protein of claim 62 wherein the tat sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 10, 27 and 70.

64. The fusion protein of claim 58 wherein the organellar targeting sequence comprises a mitochondrial targeting sequence.

65. The fusion protein of claim 64 wherein the mitochondrial targeting sequence comprises an amino acid sequence as set forth in SEQ ID NO:69.

66. The fusion protein of claim 58 wherein the IF1 polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS:12, 13, 29, 63, 64, 65, 66 and 67.

67. The fusion protein of claim 58 wherein the optional epitope tag comprises a polyhistidine tag having an amino acid sequence as set forth in SEQ ID NO:68, the cellular transport sequence comprises a tat sequence as set forth in SEQ ID NO:70, the organellar targeting sequence comprises a mitochondrial targeting sequence as set forth in SEQ ID NO:69, and the IF1 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:29.

68. The fusion protein of claim 58 wherein the fusion protein comprises an amino acid sequence as set forth in SEQ ID NO:71.

69. A fusion protein comprising (i) an optional epitope tag, which is fused to (ii) a cellular transport sequence, which is fused to (iii) an IF1 polypeptide.

70. The fusion protein of claim 69 wherein the optional epitope tag comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, and 68.

71. The fusion protein of claim 69 wherein the optional epitope tag comprises a polyhistidine tag.

72. The fusion protein of claim 71 wherein the polyhistidine tag comprises an amino acid sequence as set forth in SEQ ID NOS:1 or 68.

73. The fusion protein of claim 69 wherein the cellular transport sequence comprises a tat sequence.

74. The fusion protein of claim 73 wherein the tat sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 10, 27, and 70.

75. The fusion protein of claim 69 wherein the IF1 polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS:12, 13, 29, 63, 64, 65, 66, and 67.

76. The fusion protein of claim 58 wherein the optional epitope tag comprises a polyhistidine tag having an amino acid sequence as set forth in SEQ ID NOS:1 or 68, the cellular transport sequence comprises a tat sequence as set forth in SEQ ID NOS:10 or 70, and the IF1 polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:29.

77. A fusion protein comprising (i) an optional epitope tag, which is fused to (ii) a cellular transport sequence.

78. The fusion protein of claim 77 wherein the optional epitope tag comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:1-9, and 68.

79. The fusion protein of claim 77 wherein the optional epitope tag comprises a polyhistidine tag.

80. The fusion protein of claim 79 wherein the polyhistidine tag comprises an amino acid sequence as set forth in SEQ ID NOS:1 or 68.

81. The fusion protein of claim 77 wherein the cellular transport sequence comprises a tat sequence.

82. The fusion protein of claim 81 wherein the tat sequence is selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 10, 27, and 70.

83. A nucleic acid expression construct encoding a fusion protein according to any one of claims 53-82.

84. A host cell comprising the expression construct according to claim 83.

85. A method for producing a fusion protein comprising culturing the host cell of claim 84 and recovering said fusion protein therefrom.